Supplementary Materials:

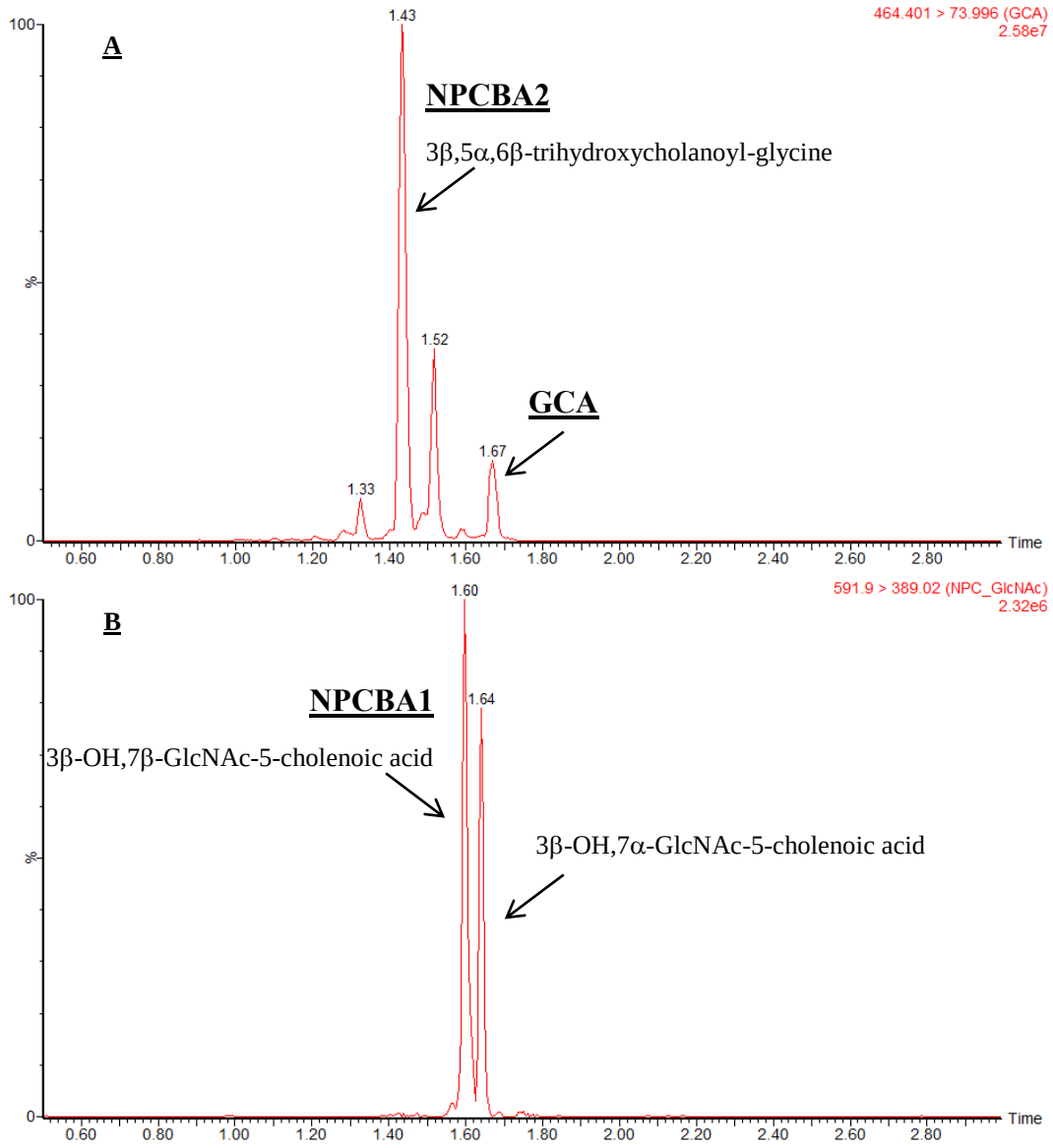
Fig. S1. Chromatographic separation between NPCBA2 and GCA (**A**) and between NPCBA1 and 3β-hydroxy,7α-*N*-acetylglucosaminyl-5-cholenoic acid (**B**).

Fig. S2. Correlation between the concentrations of NPCBA2 in plasma and DBS.

Fig. S3. Matrix effect evaluated in plasma and urine.

Table S1. NPC1 genotypes of patients included in the study.

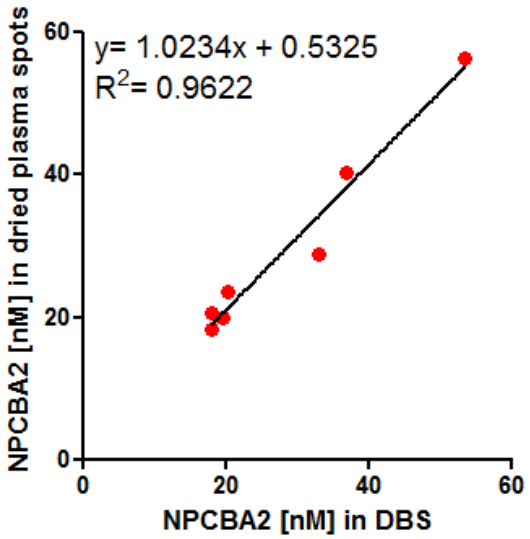
Fig. S1. Chromatographic separation between NPCBA2 and GCA (**A**) and between NPCBA1 and 3β-hydroxy,7α-*N*-acetylglucosaminyl-5-cholenoic acid (**B**).



(**A**) An example of chromatographic separation between NPCBA2 and glycocholic acid (GCA) in a urine sample from a positive NPC patient. SRM transition [464.4>74 m/z]. (**B**) Example of chromatographic separation between NPCBA1 (3β-hydroxy,7β-*N*-acetylglucosaminyl-5-

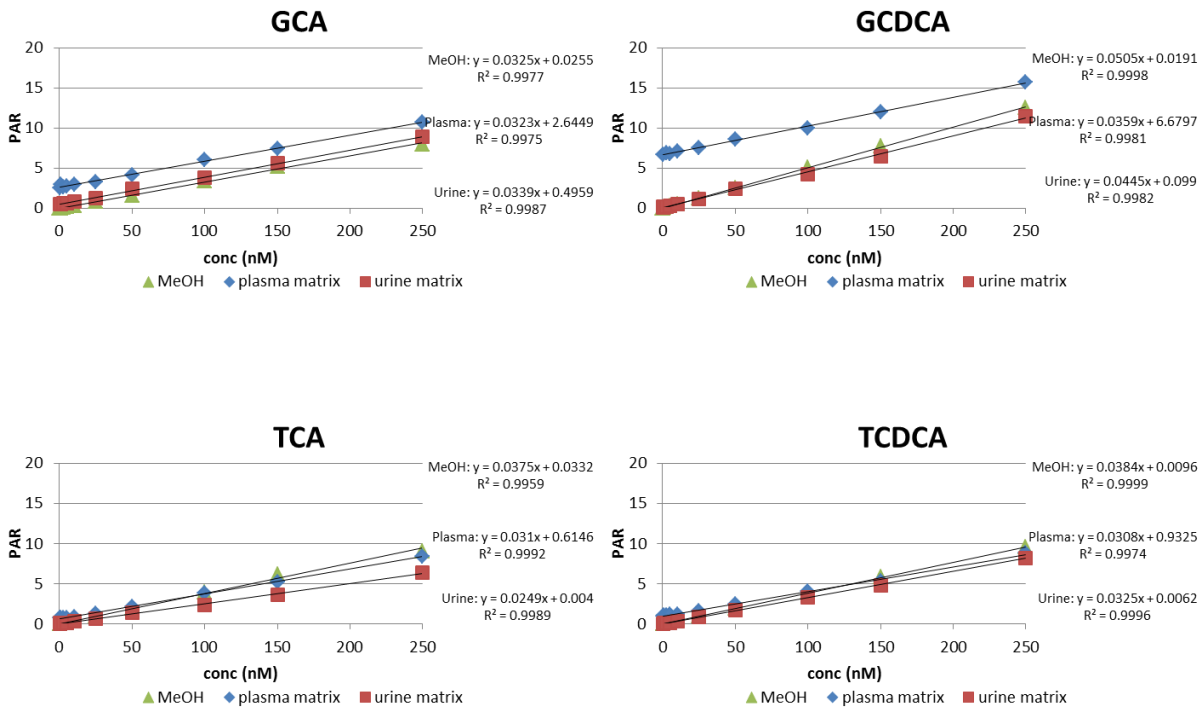
cholenoic acid) and 3β-hydroxy,7α-*N*-acetylglucosaminyl-5-cholenoic acid in a urine sample from a patient with 3β-HSDH deficiency. SRM transition [591.9>389.0 m/z].

Fig. S2. Correlation between the concentrations of NPCBA2 in plasma and DBS.



Correlation between the amount of NPCBA2 extracted from 10 µL plasma spot and 6 mm dried blood spot, estimated to contain 12.4 µL of blood (*32*).

Fig. S3. Matrix effect evaluated in plasma and urine.



Matrix effect, calculated for the main bile acids (glycocholic acid, glycochenodeoxy cholic acid, taurocholic acid and taurochenodeoxycholic acid) in plasma, urine and methanol.

Table S1. *NPC1* genotypes of patients included in the study.

Protein amino acid change	DNA nucleotide change
I1061T, F1167C	3182T>C, 3499T>G
Y825C, A105T	2474A>G, 315G>A
fs(exon12), IVS9	1920delG, 1554-1009G>A
hom. A470P and I837V	3 and 4 possible changes
hom. Q116Qfs	hom. 350delAG
F842L, F1221SfsX20	6 possible changes, 3662delT
P237S, V371X	709C>T, 11112delT
R404Q, S954L, T375A (variant)	1211G>A, 2861C>T, 1123A>G
R404Q, P1007A	1211G>A, 3019C>G
G46V, P691L	137G>T, 2072C>T
P691S, F760Sfs	2071C>T, 2279-2281TCTdel
A732Vfs, Y825C	2195insT, 2474A>G
S734I, S954L	2201G>T, 2861C>T
D771Vfs , G992R	2312_2315delACTT, 2974 G>C
Y825C, 97dupC or 97insC	2474A>G, 289_291dupTGT
F842L, T933I (also NPC2 mutation)	2524T>C, 2798C>T, 442-4A>C(NPC2)
P887L, L1247fs(exon24)	2660C>T, 3741-3744delACTC
A926V, P1007A	2777C>T, 3019C>G
S954F, P1007A	2819C>T, 3019C>G
S954L	2861C>T, IVS23+1G>A
S954L, I1061T	2861C>T, 3182T>C
R978C, IVS21-2A>G	2932C>T, 3246-2A>G
I1061T, H1016R	3047A>T, 3182T>C
A1023Sfs*15, G992R	3066_3073delinsT, 2974G>C
T1036M, F1207S	3107C>T, 3620T>C
I1061T, R1186G	3182T>C, 3556C>G
I1061T, R404Q	3182T>C, 1211G>A
I1061T, S954L	3182T>C, 1628C>T
I1061T, P691Q	3182T>C, 2072C>A
I1061T, D948N	3182T>C, 2842G>A
I1061T, 10 bp deletion in exon 19 at codon 962=fs(exon19)	3182T>C, 2884-93delinsATCACTGACC
I1061T, P1007A	3182T>C, 3019C>G
I1061T, T1036M	3182T>C, 3107C>T
I1061T, I1094T	3182T>C, 3281T>C
I1061T, E1188*	3182T>C, 3562G>T
I1061T, fs(exon24)	3182T>C, 3742_3745delCTCA
I1061T, K142Rfs	3182T>C, 423_424dupGA
I1061T, P237S, IVS12+5 G>C	3182T>C, 709C>T, 1947+5G>C

G1146V	3439G>T, 3742_3745delCTCA
hom. G1162A	hom. 3485G>C
V1165M, L1247fs(exon24)	3493G>A, 3741-44delACTC
T137M, S667L	410C>T, 20000C>T
T137M, I1061T	410C>T, 3182T>C
N222S, C468G	665A>G, 4 possible changes
N222S, I1061T	665A>G, 3182T>C
C247Y, P401T	740G>A, 1201C>A
G248V, fs (exon 22)	743G>T, 3410_3411 insA
hom I1061T	hom 3182T>C
hom. S734I	hom. 2201G>T
I1061T, V1141G	3182T>C, 3422T>G
I1061T	3182T>C, C3591+4del
I1061T, E1189G	3182T>C, 1189A>G
I1061T, R1059Q	3182T>C, 3176G>A
I1061T, R934Q	3182T>C, 2801G>A
hom. R518W	hom. 1553G>A
R518W, S95F	1553G>A, 284C>T